REMARKS

Claims 1-3 and 5-9 are pending. The Examiner has withdrawn claims 1-3 and 5-7. Applicants herein and add new claims 10-14. Support for the amendments and the new claims can be found in the specification and claims as filed. Support for new claim 10 can be found, for example, in claims 1, 3, 5, and 8. Support for new claim 11 can be found, for example, in the specification at page 5, line 31 to page 6, line 4. Support for new claims 12 and 13 can be found, for example, in claim 2. Support for new claim 14 can be found, for example, in claim 9. No new matter has been added. Applicants respectfully request entry of the new claims.

Rejections Under 35 U.S.C. § 103(a)

Claims 8 and 9 were rejected under 35 USC103(a) as allegedly obvious in light of Trowern *et al.* (US patent 6,162,903). The Examiner asserted that Trowern *e al.* discloses isolation of L protein and use in a bioassay. The Examiner conceded that Trowern *et al.* does not disclose the recited amino acid modifications of the rejected claims. The Examiner asserted it would have been obvious to use the L protein of Trowern *et al.* on a solid support to isolate an immunoglobulin.

Applicants respectfully submit that Trowern *et al.* does not render obvious the subject matter claimed. Accordingly, Applicants respectfully request that the outstanding rejection be withdrawn.

The present claims are directed to specific immunoglobulin light-chain binding proteins based on protein L. Protein L is known to have the ability to bind the light-chains of immunoglobulins and may be used therefore in their purification. For example, protein L attached to a solid support may be used to bind the κ-chain of immunoglobulins

which are subsequently eluted from the support. Conventionally, however, elution requires relatively harsh conditions.

The present claims are directed specifically to modified light-chain binding domains of protein L that are not disclosed in Trowern *et al.* Applicants have identified modified proteins comprising a substitution at one or more specific positions corresponding to positions 39, 53 or 57 in SEQ ID NO: 1 or insertion of an amino acid between positions 59 and 60. Applicants have found that such modification leads to a substantial increase in the dissociation constant (Kd). Thus, the modified binding proteins of the invention as presently claimed possess a dissociation constant of 400 nM or more at pH 8 as recited in the claims. This may be compared with the dissociation constant for wild type protein L which was determined to be approximately 112 nM (see page 16, lines 19-23 of WO 00/15803).

The modified proteins of the invention as presently claimed thus demonstrate a substantially increased dissociation constant as compared to wild type antibody binding domains. In consequence, the modified protein L domains of the invention as presently claimed allow purification of immunoglobulin under milder conditions than those required with wild type domains; the immunoglobulin may be eluted from a protein L solid support under milder conditions. This is demonstrated by the studies on affinity chromatography at pages 18-19, and in particular the results in Table 2, which show that milder changes in pH and in concentration of KCl were required to dissociate the immunoglobulin κ-chain from a protein L column using a variant of protein L substituted at the 53 position as compared to the wild type (or a variant substituted at 64 which does not substantially affect the dissociation constant).

The present application identifies substitutions at positions 39, 53 and 57 and insertion between positions 59 and 60 which markedly affect the binding affinity. The

data presented in the application is supportive of this. Accordingly, the application makes available a class of immunoglobulin light-chain binding proteins which can reasonably be predicted to possess substantially increased dissociation constant compared to the wild type binding domain. These allow the use of milder conditions for elution of antibodies from a protein L-solid support.

As has been acknowledged by the Examiner, Trowern *et al.* differs from the claimed subject matter in that the L protein-derived immunoglobulin binding proteins disclosed lack the recited amino acid modifications of the claims. Applicants submit that Trowern *et al.* does not disclose, teach, or suggest the invention as presently claimed. Applicants submit that there is nothing in Trowern *et al.* which would suggest to a person of ordinary skill in the art the specific amino acid modifications of the invention as presently claimed. Still less is there anything in Trowern *et al.* to suggest that such modifications increase the dissociation constant or provide advantages in terms of antibody purification. Indeed, Trowern *et al.* does not suggest any type of modification which might be made to protein L to alter its dissociation constant. No specific variants of protein L binding domains are disclosed Trowern *et al.*

Prima facie therefore Trowern *et al.* does not disclose, teach, or suggest the specific immunoglobulin light-chain binding proteins of the invention as presently claimed. Trowern *et al.* does not provide a person of ordinary skill in the art with any incentive to arrive at the specific mutated immunoglobulin light-chain binding proteins of the invention as presently claimed. Still less does the cited reference provide a person of ordinary skill in the art with any reasonable expectation that such proteins would possess the specific antibody binding properties Applicants have found. Nor does the cited reference suggest a method of isolating an immunoglobulin using a solid support having such a protein bound thereto.

The claimed subject matter would not therefore have been obvious to a person of ordinary skill in the art in the light of Trowern *et al.* Accordingly, Applicants respectfully request reconsideration and withdrawal of the claim rejections based on Trowern *et al.*

Conclusion

In view of the foregoing amendments and remarks, reconsideration and allowance are respectfully requested.

No fee other than the enclosed fee for a two-month extension of time is believed to be due with respect to the filing of this response. If any additional fees are due, or an overpayment has been made, please charge, or credit, our Deposit Account No. 11-0171 for such sum.

If the Examiner has any questions regarding the present application, the Examiner is cordially invited to contact Applicant's attorney at the telephone number provided below.

Respectfully submitted,

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